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Latitudinal Differences in Thermal Tolerance among Microscopic Sporophytes of the Kelp *Lessonia nigrescens* (Phaeophyta: Laminariales)[1]

ENRIQUE A. MARTÍNEZ[2]

ABSTRACT: The strong temperature increase during the 1982/1983 El Niño event caused local extinction of many species in large coastal zones of northern Chile and Peru. One brown algal species affected by massive mortality was the intertidal kelp *Lessonia nigrescens* Bory, with a latitudinal distribution from Cape Horn (55° S) to Peru (12° S). Between extreme localities of this distribution, mean annual seawater temperatures may differ by around 10°C. After the massive mortality of 1982/1983, some populations survived in a few localities of northern Chile, such as Iquique (20° S). I tested the hypothesis that these populations represent thermal ecotypes. Those from the north, close to the El Niño–impacted zone, should tolerate higher temperatures than southern populations. Microscopic sporophytes, cultivated from spores of plants collected in localities at the north, center, and south of Chile, were subjected to three temperature regimes. Two of them included the same average temperature, but different extreme values. Comparisons of thermal tolerance in the microscopic progeny from plants of the three Chilean localities showed that, at higher incubation temperatures, central and northern thermal ecotypes do have higher survival and growth rates than the ecotypes from the south. At lower incubation temperatures, the growth trend was reversed. Also, as suggested in the literature, sporophytic juveniles seem less tolerant than gametophytic microthalli. However, the differences in tolerance between northern and southern thermal ecotypes do not fully explain the survival of high seawater temperatures such as those of the 1982/1983 El Niño event by the northern populations.

TOLERANCE TO EXTREME temperatures among algal populations is crucial in establishing local and phytogeographic boundaries (Pakker et al. 1995). The distribution of brown algae along the South American Pacific coast is based on such tolerances, especially in determining the upper and lower survival limits of gametophytic microthalli, which seem more tolerant than the alternate sporophytic phase (Breeman 1988, Peters and Breeman 1993). The higher tolerance of microthalli has been suggested as favoring the dispersal of amphioceanic brown algal species across the Tropics (Peters and Breeman 1992).

In brown algae, except in some species such as *Ectocarpus siliculosus* (Dillw.) Lyngb., tolerance to high temperatures is less variable than tolerance to lower temperatures (Breeman 1988, Lüning and Freshwater 1988). Upper survival limits are important for species of the Pacific coast of North America and, particularly, South America, where natural episodic increases of seawater temperatures, attributed to El Niño events (El Niño Southern Oscillation [ENSO]), affect large geographical areas (Quinn et al. 1987). In the ENSO event of 1982/1983, northward and southward incursions of water masses with extreme high temperatures caused massive mortality of laminarian species in both hemispheres, resulting in local extinction of

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[2] Departamento de Ecología, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile (fax: 56-02-6862621; e-mail: eemartin@bio.puc.cl).

kelps along hundreds of kilometers of shoreline (Gunnill 1985, Tegner and Dayton 1987, Castilla and Camus 1992). In South America massive mortality mainly affected populations of the intertidal kelp *Lessonia nigrescens* Bory (Tomicic 1985). The latitudinal distribution of this species extends over 4000 km, from Cape Horn (55° S) to the Peruvian coast (12–14° S) (Ramírez and Santelices 1991, Peters and Breeman 1993).

After the massive mortality of *L. nigrescens* of 1982/1983, some populations survived at a few localities in northern Chile, such as Iquique at 20° S (Soto 1985). These populations are extant today, and some recolonization has taken place nearby (Camus et al. 1994). An explanation for their survival could be their tolerance to high temperatures.

Studies on temperature tolerance of brown algae from the Pacific coast of South America have stressed both interspecific differences and tolerance of gametophytic microthalli, apparently more resistant than sporophytic individuals (Peters and Breeman 1993). Ecotypic differences are important in the thermal tolerance of some brown algal species (e.g., *Laminaria saccharina* (L.) Lamour. and *Ectocarpus siliculosus* (Dillw.) Lyngb., which differ in growth and survival, respectively, under high temperatures [Breeman 1988]).

In this study, using the current distribution of *L. nigrescens,* I tested the hypothesis that thermal tolerance of sporophytic microthalli may be higher in individuals of northern origin, where populations are probably subjected to episodic ENSO events. For this purpose, thermal tolerance was compared among cohorts of microscopic sporophytic progeny from *L. nigrescens* collected at three sites (20° S, 33° S, and 41° S) over its distributional range. Microscopic individuals were subjected to three thermal conditions, and survival and growth of microscopic sporophytes were compared.

MATERIALS AND METHODS

Study Sites

Sampling sites along the Chilean coast (Figure 1) were Aguadita, in the north (20° 36′ S, 70° 10′ W), 40 km south of Iquique; Las Cruces, in the center (33° 30′ S, 71° 38′ W); and Pucatrihue, in the south (40° 27′ S, 73° 46′ W). Reproductive fronds of *L. nigrescens* from each site were collected from plants in the low intertidal.

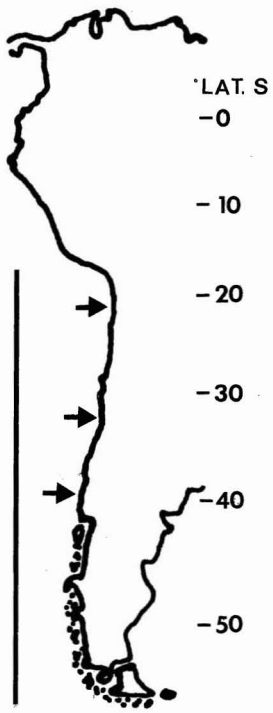

FIGURE 1. Pacific coast of South America and distribution of *Lessonia nigrescens* (vertical line), modified from Peters and Breeman (1993). Arrows show collection sites of mature fronds of *L. nigrescens* used in experiments on thermal tolerance.

Annual Regime of Sea Surface Temperatures

Temperature records were obtained daily from the marine research stations closest to each sampling site. At Las Cruces and Iquique, records of the temperature of surface seawater were measured at 1-month intervals, from 4 January to the end of November 1993. Data were taken every 3 days, at noon, on the same dates. In the absence of similar records for the southern locality of Pucatrihue, only data available for surface temperatures measured daily at Mehuín

(39° 26′ S, 73° 13′ W) were used. These data corresponded to the same months mentioned above, but they were collected during 1988. The monthly means of surface temperatures for the three localities were compared using a Kruskal-Wallis test (Siegel and Castellan 1988), because different transformations did not result in normally distributed data.

Mortality and Growth of Microscopic Plants at Different Temperatures

In November 1993, adult plants were collected from Aguadita (14 November 1993), Las Cruces (15 November 1993), and Pucatrihue (27 November 1993). Mature sori from 10 plants were collected and held in the laboratory at ambient seawater temperature. They were placed on tissue paper at room temperature (16°C) for 2 hr, rehydrated in 400 ml of 0.45-μm-filtered seawater, and agitated at 120 rpm on a shaker (Junior Orbit). Spores were released and each suspension was diluted with filtered seawater to 500,000 spores ml^{-1}. This spore suspension was poured into 500-ml plastic containers for 12 hr to allow spores to settle on three slides placed on the bottom of the containers. Then the remaining suspension was replaced by 500 ml of filtered seawater, enriched with sodium nitrate (400 nM, final concentration) and sodium phosphate (20 μM, final concentration). Light was provided at a 12:12 photoperiod, with two circular 32W fluorescent tubes (Hitachi FC12T9/D) providing a photon flux density of 40 μmol $m^{-2}sec^{-1}$. Thermometers were placed in the incubation containers and temperature was recorded during light and dark periods to facilitate control of the conditions in the incubation chambers, as described below.

Before the experiments the settled spores were acclimated in separate containers at 15°C for 3 weeks. During this period, gametophytes from each locality grew to maturity and reproduced, producing the next generation of sporophytes.

After the acclimation period, the three incubation conditions for microscopic sporophytes were as follows: treatment 1 (control) with lower mean temperature (ca. 11°C) and treatments 2 and 3 with similar high mean temperatures (ca. 20°C) but different extremes. Treatment 2 differed from treatment 3 in having a broader range but lower extreme high temperature (Table 1). These conditions were replicated three times. Mean temperatures of the two warmer treatments were similar ($P > 0.05$, Tukey a posteriori test), but different from that of the control ($F = 96.707$; $df = 2,35$; $P < 001$, one-way analysis of variance [ANOVA]). To avoid potentially lethal variations, 2 hr were programed from the minimum temperature to the maximum in the light phase and a similar decrease in the dark phase.

TABLE 1

INCUBATION CONDITIONS FOR THREE TEMPERATURE TREATMENTS APPLIED TO MICROSCOPIC PROGENY OF *Lessonia nigrescents* PLANTS FROM THE NORTH (AGUADITA), THE CENTER (LAS CRUCES), AND THE SOUTH (PUCATRIHUE) OF CHILE

	TEMPERATURE VARIABLES (°C)				
TREATMENT	MEAN	SE	*n*	MIN.	MAX.
1	10.9	0.47	13	9.0	16.0
2	19.0	0.75	12	13.5	22.0
3	20.8	0.38	13	20.0	24.0

Size sampling of microscopic sporophytes on each slide was accomplished by taking nine photographs at regular distances on a 5-cm transect under a light microscope (Nikon-Biophot). Each photograph included a sampling area of 0.077 mm^2.

Microscopic sporophytes were then subjected for 12 days to the three temperature treatments, and a second sampling of the slides was conducted at the end of the period. Mortality was estimated by comparing the number of dead (bleached) and living sporophytes at the end of the 12-day experiment. The size of microscopic juveniles was measured on magnified images of both sets of samples, and growth was estimated as the percentage increase in length in comparison with the mean initial sporophyte size. Arcsine-transformed growth percentages of surviving microscopic progeny from the three different localities and treatments were compared using a two-way ANOVA (Statgraphics 1988).

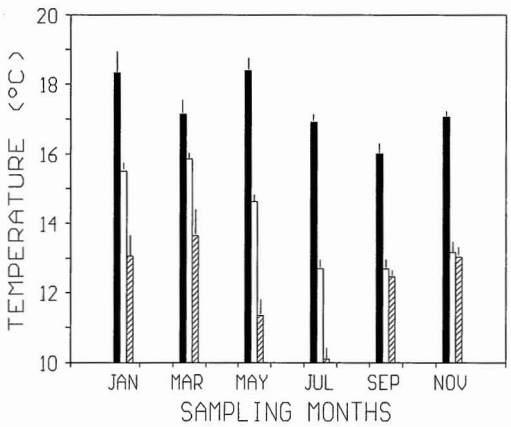

FIGURE 2. Mean monthly temperatures (±1 SD) of surface seawater, measured in northern (Iquique, solid bars), central (Las Cruces, open bars), and southern Chile (Mehuín, hatched bars).

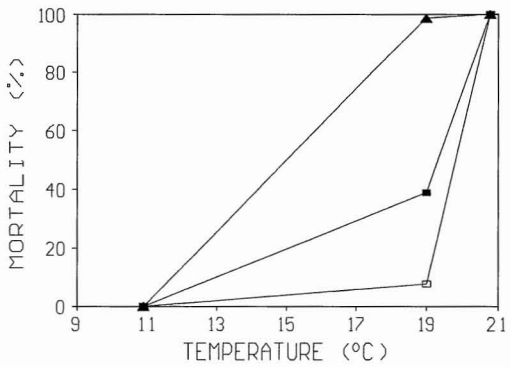

FIGURE 3. Mean mortality under three temperature treatments (see Table 1) of microscopic progeny of *Lessonia nigrescens* from three localities along the Chilean coast: the north (Aguadita, solid squares), the center (Las Cruces, open squares), and the south (Pucatrihue, solid triangles).

RESULTS

Annual Regime of Sea Surface Temperatures

Mean sea surface temperatures were significantly higher in Iquique than at the two other localities (Las Cruces and Mehuín) for all sampled months (Kws > 15.3, $P < 0.001$, Kruskal-Wallis test); mean values in Las Cruces were between those of the two extreme localities (Figure 2). At Iquique, during the sampling period, maximum mean monthly temperatures were never higher than 18.4°C or below 16°C. The only nonsignificant differences in temperatures were those between Mehuín and Las Cruces in September and November (Tukey a posteriori test).

Mortality and Growth of Microscopic Plants at Different Temperatures

The total number of sporophytes per slide from each locality and treatment (1–3) was as follows: Aguadita: 1, 142; 2, 170; 3, 49; Las Cruces: 1, 127; 2, 105; 3, 37; Pucatrihue: 1, 80; 2, 65; 3, 21. When incubated at a mean temperature of 10.9°C (treatment 1), no mortality was observed for microscopic plants of any locality (Figure 3). However, progeny of plants from all three localities underwent some degree of mortality when incubated at a mean temperature of 19°C (treatment 2). Under this treatment, mortality was particularly high (98.5%) for the microscopic progeny from the southernmost site, Pucatrihue (Figure 3). After 12 days under higher minimum and maximum temperatures (treatment 3), all plants were dead (Figure 3).

The surviving microscopic plants incubated at a mean temperature of 19°C (treatment 2) and all of those incubated at a mean of 10.9°C (treatment 1) did grow in the 12-day incubation period. The mean initial size of sporophytes from each locality was 17.6 μm (SD = 2.5, $n = 40$) for Iquique, 18.0 μm (SD = 3.2, $n = 40$) for Las Cruces, and 15.4 μm (SD = 2.5, $n = 15$) for Pucatrihue. Growth ranged from 30 to 60%, and greater differences between localities were observed at 19°C than at 10.9°C (Figure 4). The ANOVA (Table 2) showed that growth rates were significantly different according to locality (higher growth for sporophytes from the north) and according to the interaction of locality and temperature. Sporophyte growth from northern and central localities only differed at 10.9°C (Scheffe a posteriori test), with greater growth in juveniles from the north. As expected, at this lowest incubation temperature, growth in juveniles from the

south was significantly greater than in those from the center, but, surprisingly, not different from those from northern Chile (Scheffe a posteriori test, Figure 4). This phenomenon probably contributed to the significance of the interaction factor (Table 2). Conversely, at the warmer temperature, microscopic sporophytes from the colder, southern site had the least growth (Scheffe a posteriori test, Figure 4).

DISCUSSION

Typically, the mean monthly temperatures of surface seawater increase from southern (41° S) to northern Chilean localities, but do not exceed 20°C at Iquique (20° S). This pattern may partially explain the high mortality of microscopic juveniles of *Lessonia nigrescens* when exposed to thermal regimes. Mortality was particularly high in the progeny from the southernmost locality, with about the same effects under treatments 2 and 3 (with the same mean temperatures). Temperature maxima higher than 22°C (the highest value in treatment 2) and up to 24°C were associated with the higher mortality of microscopic sporophytes in treatment 3. The high mortality of microscopic sporophytes from the southern locality of Pucatrihue in treatments 2 and 3 indicates much less tolerance to long-term elevated temperatures for the progeny of plants from these naturally cold environments. This effect may also be seen at even shorter exposures to high temperatures. The experimental period was sufficient to reveal this threshold to thermal tolerance. Also, progeny from the south, surviving to a mean incubation temperature of 19°C, showed highly reduced growth compared with that shown at 10.9°C. This further suggests their lesser tolerance to long-term exposure to high temperatures.

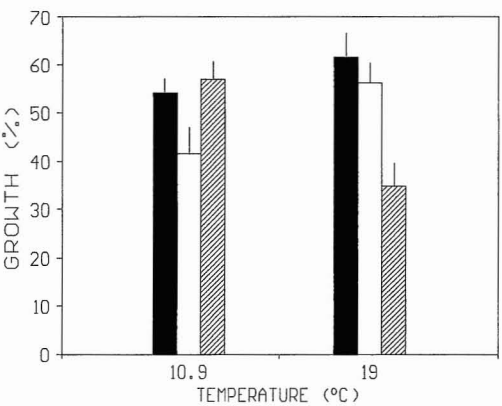

FIGURE 4. Growth (percentage in 12 days of incubation) of microscopic sporophytic progeny of *Lessonia nigrescens* from three localities along the Chilean coast: the north (solid bars), the center (open bars), and the south (hatched bars). Vertical lines indicate 95% confidence intervals.

These differences in mortality and growth in plants from extreme localities of the latitudinal distribution suggest the presence of different reaction norms (sensu Thompson 1991). The expression of these norms (detected in the sporophytic progeny beyond the intermediate gametophytic phase) also suggests that such responses are inheritable

TABLE 2

RESULTS OF MULTIFACTOR ANOVA FOR GROWTH RESPONSES IN THE SPORPHYTIC PROGENY OF *Lessonia nigrescens* FROM THREE LOCALITIES, EVALUATED UNDER TWO INCUBATION TEMPERATURES, IN COMPARISON WITH CONTROL REGIMES

SOURCE OF VARIATION	SUM OF SQUARES	df	MEAN SQUARE	*F*	*P*
Main effects	0.899	3	0.299	6.6	0.0002
Locality	0.772	2	0.386	8.5	0.0002
Temperature	0.128	1	0.128	2.8	0.0949
Two-factor interactions	1.286	2	0.643	14.1	<0.0001
Locality and temperature	1.286	2	0.643	14.1	<0.0001
Residual	27.028	593	0.046		
Total	29.214	598			

features in populations of *L. nigrescens* from different latitudinal distribution.

Peters and Breeman (1993) showed that even haploid gametophytic microthalli of *L. nigrescens* from southern Chile (ca. 39° S) survived after exposure to a temperature of 24.4°C for 2 weeks. This period is 2 days longer than the one used in my study (12 days), in which I found higher mortality of microscopic sporophytes at a mean temperature around 20°C, occasionally peaking to 24°C. All this evidence confirms that sporophytes are less tolerant than gametophytic microthalli, as suggested by Breeman (1988).

The results of this study indicate that microscopic sporophytes from northern sites are more tolerant to high temperatures than those from southern, colder latitudes. However, for several reasons, this higher tolerance of northern plants does not fully explain the survival of populations extant today, after the strong ENSO event in 1982/1983. The possibility that even more resistant gametophytic microthalli could contribute to such survival might also be misleading. Gametophytic stages have a short life span, probably shorter than the several weeks that high temperatures (above 24°C) occurred during the strong ENSO event of 1982/1983. In fact, when haploid spores of *L. nigrescens* are released, they settle and may produce new sporophytes within a couple of days, and female individuals can be fertilized at the single-cell stage, before the settled spore undergoes mitosis (pers. obs.). The short period between spore release and fertilization of gametophytes would likely have caused the less-tolerant young sporophytes to be exposed almost immediately, for several weeks, to the high temperatures of the ENSO event (ca. 30°C in 1982/1983). Further, the germination potential of nonsettled spores remaining in the plankton for 1 or 2 days is rapidly reduced (Hoffmann and Camus 1989). Even if those spores do germinate, only 1 day in a spore suspension reduces their adhesive capacity (pers. obs.). Consequently, during ENSO events, the less-tolerant microscopic sporophytes would be quickly exposed to critical high temperatures.

Compared with that of southern microscopic juveniles, the experimentally determined tolerance of northern microscopic sporophytes of *L. nigrescens* was not high enough to account for the survival of some populations to the ENSO event of 1982/1983. Further complicating this issue is that ecotypic differences within northern populations in 1982/1983 may have been even weaker than those observed today between northern and southern populations.

Thus, another explanation for the survival of some populations is that they were not actually exposed to high temperatures and somehow remained isolated from increases in surface seawater temperature. For example, colder water masses remaining close to shore during the ENSO event may have allowed survival of today's extant populations. These masses could have remained from previous upwelling events or they might have been cooled by waters emerging from underground. This last phenomenon has been reported in other parts of the world, such as Port Miou, France (Potié 1973, Scanvic 1983), and also for some localities of northern Chile, included Aguadita, where sifting subterranean waters are associated with transverse geological faults (L. Velozo, pers. comm.). Nutrient depletion is usually associated with ENSO events and the interaction with high temperatures seems to be the critical factor causing massive algal mortality (Gerard 1997). In December 1982, at least in some locations in northern Chile, nutrients such as nitrate, nitrite, and phosphate occurred in higher concentrations at the sea surface than in other ENSO events (Díaz 1984). Thus, isolated areas of the coast might have had lower temperatures and/or enough nutrients to allow these algae to withstand the critical conditions that occur during these events.

The small difference in mortality between the progenies of plants from north and central Chile suggests that the great distance and different temperature regimes between these localities is not reflected in noticeable adaptation, at least as revealed in physiological properties. In other brown algal species such differences may be expressed even at very short distances, as are those in strains of

Scytosiphon lomentaria (Lyngbye) Link, where extents of tolerance to temperature and salinity are highly different, even over very small spatial scales (Kristiansen et al. 1994). Thus, *L. nigrescens* should not be considered a potential indicator species of surface-water heating during global warming events in northern Chile. The disappearance of populations might reflect slight variations in the temperature of water masses, but occurring over a very short period of time. Conversely, the time scale at which global warming might occur is considerably longer (Lubchenco et al. 1993), and some level of physiological accommodation would be expected. But phenotypic acclimation is possible on very short time scales.

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